UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: **2,4-Dichlorophenoxyacetic acid:** Industry Task Force II on 2,4-D Research Data.

FROM: Jess Rowland, M.S. Toxicologist Jess Rowland 7/3/91
Section II, Toxicology Branch II
Health Effects Division (H7509C)

TO: Lois Rossi
Product Manager (50)
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THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 7/3/91
Section II, Toxicology Branch II
Health Effect Division (H7509C)
and
Marcia Van Gemert, Ph.D., Chief Marcia Van Gemert 7/9/91
Toxicology Branch II
Health Effects Division (H7509C)

TASK IDENTIFICATION: Submission No. **8390397** Case No. **818706**

HED Project No. **1-0621** Caswell No(s): **315; 315- AS and 315-O**

Registrant: Industry Task Force on 2,4-D Research Data

ACTION REQUESTED: In-depth review of the toxicology data.

RESPONSE: The registrant submitted **21-day dermal irritation and toxicity studies** (Range-Finding and Full Scale) in rabbits **with 2,4-D acid, 2,4-D-2 ethylhexyl ester, and the dimethylamine salt of 2,4-D to satisfy** Guideline 82-2, and a **developmental toxicity** in rabbits **with 2,4-D acid** to satisfy Guideline 83-3.

The **21-day dermal toxicity studies** are classified as **Core Guideline** and satisfies the testing requirements for dermal toxicity study (82-20 in rabbits.

The **developmental toxicity study** is classified as **Core Minumum** and satisfies the testing requirement for a developmental toxicity study (83-3) in rabbits.

A separate Data Evaluation Report (DER) for the above reference studies is attached. The results of each study is as tabulated below:

A. 21-Day Range Finding Studies

Compound	2,4-D Acid	2,4-D EH Ester	2,4-D DMA
Caswell No.	315	315 AS	315 O
MRID No.	417353-01	417353-02	417353-03
Study No.	HLA 2184-106	HLA 2184-107	HLA 2184-108
Report Date	July 10, 1990	July 25, 1990	August 2,1990
Dose Levels Tested (mg/kg/day)	10, 30, 300, 1000	16.3, 48.8, 162.8, 488.3, 1627.5	18, 54.1, 180.2, 540.5, 1801.8
Dose Levels Selected for Full Scale Studies	10, 100,1000 mg/kg/day	16.3, 162.8, 1627.5 mg/kg/day	18, 54.1, 180.2 mg/kg/day

B. 21-Day Toxicity Studies

Compound	2,4-D Acid	2,4-D EH Ester	2,4-D DMA
Caswell No.	315	315 AS	315 O
MRID No.	417353-04	417353-05	417353-06
Study No.	HLA 2184-109	HLA 2184-110	HLA 2184-111
Report Date	October 16,1990	July 30, 1990	August 9, 1990
Dose Levels Tested (mg/kg/day)	10, 100, 1000	16.3, 162.8, 1627.5	18,180.1, 540.5
NOEL: Dermal irritation	1000 mg/kg/day	16.3 mg/kg/day	18 mg/kg/day
NOEL: Systemic	1000 mg/kg/day	1627.5 mg/kg/day	540.5 mg/kg/day
LOEL: Dermal irritation	Not established	162.5 mg/kg/day	180.1 mg/kg/day

C. Developmental Toxicity Study

End Point	NOEL	LOEL
Maternal	30 mg/kg/day	90 mg/kg/day
Developmental	90 mg/kg/day	>90 mg/kg/day

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Section II,Tox.Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel 7/3/91
Head, Section II, Tox.Branch II (H7509C)

DATA EVALUATION REPORT

Study Type: 21-Day Dermal Toxicity (Range-Finding Studies).

Study Identifications:

Compound	2,4-D Acid	2,4-D EH Ester	2,4-D DMA
Caswell No.	315	315-AS	315-O
MRID No.	417353-01	417353-02	417353-03
Study No.	HLA 2184-106	HLA 2184-107	HLA 2184-108
Report Date	July 10,1990	July 25,1990	August 2,1990

Sponsor: Industry Task Force II on 2,4-D Research Data.

Testing Laboratory: Hazleton Laboratories America, Inc.

Study Author: Gene E. Schulze, Ph.D., D.A.B.T.

SUMMARY/CONCLUSION:

Compound	Dose Levels (mg/kg/day)	Treatment	Doses Selected for Full-scale study (mg/kg/day)
2,4,D- Acid	10, 30, 100, 300, 1000	6 hr/day, 7 days/week for 21 days	10, 100, and 1000
2,4-D EH Ester	16.3, 48.8, 162.8, 488.3, 1627.5	6 hr/day, 7 days/week for 21 days	16.3,162.8, and 1627.5
2,4-D DMA	18, 54.1, 180.2, 540.5, 1801.8	6 hr/day, 7 days/week for 21 days	18, 54.1, and 180.2

1. **21-Day Dermal Irritation and Dermal Range Finding Study in Rabbits with 2,4-Dichlorophenoxyacetic acid (MRID No.417353-01).**

I. INTRODUCTION

A **range-finding study** was conducted **with 2,4-D acid** in rabbits to select dose levels for a full scale 21-day dermal irritation study.

II. MATERIALS & METHODS

Test Material:	**2,4-D acid** (lot No.909; Purity: 96.1%).
Test Animals:	Male and female Hra:(NZW)SPF rabbits.
Groups Size:	1 male and 1 female.
Dose Levels:	10, 30, 100, 300, and 1000 mg/kg/day
Treatment:	Test material (beige powder) was applied topically on the dorsal shaved area (approximately 10% of the total body surface) of each animal and 0.5 ml of distilled water was dispersed over the compound. The test site was covered with gauze/binder/wrap.
Exposure Time:	6 hours/day, 7 days/week for 21-days
Observations:	Mortality, moribundity, clinical signs, body weights (Days 0, 7, 14, and 21), and gross pathology.

III. RESULTS

No treatment-related effects were seen on survival, clinical signs, body weight, body weight gain, and gross pathology. Except for a slight erythema in one male rabbit at the 10 mg/kg/day on days 2 and 3, no compound-related dermal irritation was observed at the dosage levels tested.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with **2,4-D acid** were: 10, 100, and 1000 mg/kg/day (limit dose).

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

2. 21-Day Dermal Irritation and Dermal Range Finding Study in Rabbits with 2,4-Dichlorphenoxyacetic Acid-2-Ethylhexyl Ester (MRID No.417353-02).

I. INTRODUCTION:

A **range-finding study** was conducted **with 2,4-D EH Ester** in rabbits to select dose levels for a full scale 21-day dermal irritation study.

II. MATERIALS & METHODS

Test Material:	**2,4-D acid-2-ethylhexyl ester** (lot No.04KF54479; Purity: 98%; acid equivalent-62.8%).
Test Animals:	Male and female Hra:(NZW)SPF rabbits.
Groups Size:	1 male and 1 female.
Dose Levels:	16.3, 48.8, 162.8, 488.3, and 1627.5 mg/kg/day
Dose Volume:	0.01, 0.04, 0.14, 0.42, and 1.40 mL/kg
Treatment:	Test material (yellow liquid) was applied (neat) topically on the dorsal shaved area and the test site was covered with gauze/binder/wrap.
Exposure Time:	6 hours/day, 7 days/week for 21-days
Observations:	Mortality, moribundity, clinical signs, body weights (Days 0, 7, 14, and 21), and gross pathology.

III. RESULTS

One female rabbit at 48.8 mg/kg/day group was sacrificed moribund on Day 10 due to enteritis, a common condition seen in rabbits in laboratory housing. No treatment-related effects were seen in body weight gain, clinical signs or gross pathology at any dose level. No dermal irritation was observed at 16.3 mg/kg/day dose. Dose-related dermal irritation at 48.8, 162.8, 488.3 and 1627.5 mg/kg/day included erythema, slight edema, atonia, epidermal scaling, skin fissuring, and transient thickening. Generally, erythema scores were more severe during the first 3 days of the study and the incidence and severity of the scores appeared to be dose related. Epidermal scaling, atonia and skin fissuring were first seen at 1627.5 mg/kg/day group animals after 5 to 9 days.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with **2,4-D acid-2 EH Ester** were:16.3, 162,8, and 1627.5 mg/kg/day (limit dose).

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

3. 21-Day Dermal Irritation and Dermal Range Finding Study in Rabbits with Dimethylamine Salt of 2,4-Dichlorophenoxy-acetic acid (MRID No.417353-03).

I. INTRODUCTION

A range-finding study was conducted with 2,4-D DMA in rabbits to select dose levels for a full scale 21-day dermal irritation study.

II. MATERIALS & METHODS

Test Material:	2,4-D DMA (lot No.04FD31349; Purity: 66.18%; acid equivalent-55.5%).
Test Animals:	Male and female Hra:(NZW)SPF rabbits.
Groups Size:	1 male and 1 female.
Dose Levels:	18, 54.1, 180.2, 540.5, and 1801.8 mg/kg/day
Dose Volume:	0.02, 0.04, 0.15, 0.44 and 1.50 mL/kg
Treatment:	Test material (liquid) was applied (neat) topically on the dorsal shaved area of each animal and the test site was covered with gauze/binder/wrap.
Exposure Time:	6 hours/day, 7 days/week for 21-days
Observations:	Mortality, moriburdity, clinical signs, body weights (Days 0, 7, 14, and 21), and gross pathology.

III. RESULTS

No severe treatment-related effects were seen in rabbits receiving 2,4-D DMA at doses 18, 44.1, 180.2, or 540.5 mg/kg/day. The two high-dose (1801.8 mg/kg/day) animals found prostrate and exhibiting tremors were sacrificed on Day 2 of the study. No gross pathological changes were seen at necropsy. Dermal irritation and skin changes occurred in all treatment groups in a dose-related manner and consisted of various degrees of erythema and edema. Additional dermal changes included skin thickening, epidermal scaling, atonia, fissuring (some with bleeding), sloughing, necrosis, necrotic raised areas and brown staining of the application area.

IV. CONCLUSION

Based on these findings, the dose levels selected for the full scale 21-day dermal toxicity study with 2,4-D DMA were: 18, 54.1, and 180.2 mg/kg/day.

V. CORE CLASSIFICATION

Not applicable; range finding study to determine dose levels for a full scale study.

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SECONDARY REVIEWER: K. Clark Swentzel 7/3/91
Head, Section II, Tox.Branch II (H7509C)

DATA EVALUATION REPORT

Study Type: 21-Day Dermal Toxicity (Full-Scale Studies).

Guideline Requirement: 82-2

Study Identifications:

Compound	2,4-D Acid	2,4-D EH Ester	2,4-D DMA
Caswell No.	315	315-AS	315-O
MRID No.	417353-04	417353-05	417353-06
Study No.	HLA 2184-109	HLA 2184-110	HLA 2184-111
Report Date	October 16,1990	July 30,1990	August 9, 1990

Sponsor: Industry Task Force II on 2,4-D Research Data.

Testing Laboratory: Hazleton Laboratories America, Inc.

Study Author: Gene E. Schulze, Ph.D., D.A.B.T.

SUMMARY/CONCLUSION:

4. 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with 2,4-Dichlorophenoxyacetic Acid (MRID No. 417353-04).

Repeated dermal application **2,4-Dichlorophenoxyacetic acid** to male and female rabbits at doses 0, 10, 100, or 1000 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. 2,4-D acid was mildly irritating to the skin of rabbits inducing very slight erythema and epidermal scaling; in general, females were noted to have a higher incidence of dermal findings than males. No treatment-related effects were seen in survival, clinical signs, body weight gain, clinical, gross, or histopathology. Based on the results of this study, the no-observable-effect level (NOEL) for dermal irritation and systemic toxicity was 1000 mg/kg/day, the highest dose tested. A Lowest-Observable-Effect-Level (LOEL) was not established,

This study is classified as **Core Guideline** and satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

5. 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with 2,4-Dichlorophenoxyacetic Acid-2-ethylhexyl Ester (MRID No. 417353-05).

Repeated dermal application **2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester** to male and female rabbits at doses 0, 16.3, 162.8, 1627.5 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. 2,4-D-2-ethylhexyl ester induced dermal irritating consisted of various degrees of erythema and edema whose incidence and severity were dose dependent. Additional dermal changes included skin thickening, epidermal scaling, fissuring (some with bleeding), and atonia. No treatment-related effects were seen in survival, clinical signs, food consumption or clinical pathology. Statistically significant depressions of body weight change were seen in treated females and nonsignificant depressions were seen in treated males during study week 3. A significant increase in mean relative kidney weights was observed for males at 1627.5 mg/kg/day group; however, the biological significance of this increase is not clear due to a lack of corroborating changes in clinical chemistry parameter or histopathological changes in the kidneys. Histopathologic changes observed in the treated skin included acanthosis, hyperkeratosis, and necrotic cellular debris on the epidermal surface; the incidence and severity of these lesions were increased in both males and females at the 162.8 and 1627.5 mg/kg/day groups. No histopathological lesions were seen in either sex at the low dose (16.3 mg/kg/day). Based on the results of this study, the no-observable-effect level (NOEL) was 16.3 mg/kg/day for dermal irritation and 1627.5 mg/kg/day for systemic toxicity. The lowest-observable-effect level (LOEL) for dermal irritation was 162.5 mg/kg/day.

This study is classified as **Core Guideline** and satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

6. 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid (MRID No. 417353-06).

Repeated dermal application of Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid to male and female rabbits at doses 0, 18, 180.1, or 540.5 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. Treatment-related dermal irritation consisted of various degrees of erythema and edema; the incidence and severity being dose dependent and generally greater in females than males. Additional dermal changes, also dose dependent, included skin thickening, epidermal scaling, fissuring (some with bleeding), necrosis, sloughing, compound-like material at treatment site, and atonia. Remarkable dermal changes were seen at 180.1 and 540.4 mg/kg/day dose groups, while only occasional, very slight erythema was seen at 18 mg/kg/day group; the latter finding was not considered to be toxicologically significant.

No treatment-related effects were seen in survival, clinical signs, body weight, body weight gain, food consumption, or clinical pathology. The statistically significant increase in absolute kidney weight of male rabbits at 180.1 mg/kg/day group was not considered to be biologically significant since no changes were observed in relative kidney weight or in histopatholcgy. Histopathological changes observed in treated skin at 180.1 and 540.5 mg/kg/day groups included acanthosis, hyperkeratosis, edema, superficial crusting (inspissated serum, necrotic cells and debris on epidermal surface) and chronic active inflammation. The treated skin lesions of the low-dose animals were comparable to controls. Based on the results of this study, the no-observable-effect level (NOEL) was 18 mg/kg/day for dermal irritation and 540.5 mg/kg/day for systemic toxicity. The LOEL was 180.1 mg/kg/day for dermal irritation.

This study is classified as **Core Guideline** and satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

7. **Developmental Toxicity** (Embryo-Fetal Toxicity and Teratogenic Potential) Study of **2,4-Dichlorophenoxyacetic acid** (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits **(MRID No. 417476-01).**

2,4-D Acid was administered by gavage to presumed pregnant rabbits at 0, 10, 30, and 90 mg/kg/day in 0.5% methylcellulose. Maternal toxicity was observed in two high dose does with ataxia. One high dose doe that aborted on day 21 had decreased body weight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group. There were no significant or dose-related alterations in fetal development that could be attributed to treatment. The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to control. Based on these findings the following NOELs and LOELs were established.

End Point	NOEL	LOEL
Maternal	30 mg/kg/day	90 mg/kg/day
Developmental	90 mg/kg/day	>90 mg/kg/day

This study is classified as **Core Minimum** and satisfies the requirements for a developmental toxicity study (83-3b) in rabbits.

1. 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with 2,4-Dichlorophenoxyacetic Acid (MRID No. 417353-04).

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with 2,4-D acid.

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: 2,4-Dichlorophenoxyacetic Acid
Purity: 96.1% pure
Lot No.: 909
Description: Beige-colored powder

2. Test Animals

Species: Rabbit
Strain: Hra:(NZW)SPF
Sex: Males and females
Age at Initiation: Adult
Weight at Initiation: males - 2.1 to 2.6 kg;
females - 2.2 to 2.6 kg
Identification: Individual numbered ear tags and cage cards.
Acclimation: Approximately two weeks
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food: Certified High Fiber Rabbit Chow #5325 ad libitum
Water: Tap water ad libitum
Environment: Temperature- 70 to 79°F; Humidity- 24 to 62%
Light / dark cycles: 12 hr. light/dark cycle

3. Study Design

Group No.	No. of Animals Males	Females	Actual Test Material (mg/kg/day)[a]
1 (vehicle control)	5	5	0[b]
2 (Low-dose)	5	5	10
3 (Mid-dose)	5	5	100
4 (High-dose)	5	5	1000

[a] Based on results of a 21-day pilot range-finding study.
[b] The vehicle control group received distilled water.

4. Test Material Formulation

The test material was administered neat on a mg/kg/day basis. The test material was weighed, and subsequently sprinkled evenly on a 4- x 4-inch gauze pad and moistened with 0.5 mL of distilled water to ensure good contact with the skin.

5. Treatment

Approximately 24 hours prior to dosing, the fur was clipped from dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. The appropriate amount of the test material paste was applied uniformly over the exposure area; the control group received distilled water. The test material was held in contact with the skin for a 6-hour period each day by a gauze dressing that was secured with a Coban® wrap, and the wrap was secured with an nonirritating adhesive tape. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore a flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was gently wiped (but not washed) with dry gauze to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. Experimental Procedures

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Clinical signs	Days 0, 7, 14, and 21.
Body weight	Prior to initiation and on Days 7, 14, and 21.
Food consumption	Days 2,4,6,7,9,11,13,14,16, 18,20, and 21.
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit (HCT)	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Cell morphology
Mean cell hemoglobin (MCH)	Corrected leukocyte count
Mean cell hemoglobin concentration (MCHC)	

Clinical Chemistry

Calcium	Albumin
Inorganic phosphorus	Globulin
Chloride	Total protein
Sodium	Creatinine
Potassium	Total bilirubin
Glucose	Triglycerides
Alanine aminotransferase (ALT)	Total cholesterol
Aspartate aminotransferase (AST)	Alkaline phosphatase
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

7. Termination

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the kidneys, thyroid/parathyroid, testes with epididymides, and liver with drained gall bladder were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. Histopathology

The tissues listed above from all animals were trimmed and processed for histopathological evaluation.

9. Statistical Analyses

Appropriate statistical methods were employed for the analyses of body weights, body weight changes, food consumption, clinical pathology parameters and absolute and relative organ weight values.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories America, and the study Protocol.

III. RESULTS

1. Survival

One male rabbit at the high-dose (1000 mg/kg/day) was found dead on Day 5; death was not attributed to treatment.

2. Clinical Signs

Except for pale/languid appearance and wheezing of the male that died, no treatment-related clinical signs of toxicity were observed during the study.

3. Dermal Observations

Vehicle controls: one female showed very slight erythema on Days 17 and 18, and another female had well-defined erythema on Day 16; no dermal irritation was seen in the males.

Low dose (10 mg/kg/day): one male had very slight erythema on Day 21, and 4 of 5 females developed very slight erythema during the dosing period. Females exhibited these dermal reactions during Days 6 to 12 and 19 to 21. Epidermal scaling was observed in one female on Days 6 and 7, and in two females on Day 12; no epidermal scaling was seen in males. One female had very slight edema on Day 21; no edema was seen among males.

Mid-dose (100 mg/kg/day): Among males, very slight erythema was seen beginning on Day 6: in 1 animal during Days 6 to 9 and on Day 15; in 2 animals on Days 10, 11, 14, 16, and from Days 18 to 20; and in 4 rabbits on Day 21. No erythema was seen on Days 12, 13, and 17. Among females, very slight erythema was seen beginning on Day 1; no erythema was seen on Days 2, 3, 13, 14, and 15. This lesion was observed in 2 rabbits on Days 1, during Days 6 to 12, and during Days 16 to 21; and in 3 animals on Days 4 and 5. No edema was seen in this group. One female had epidermal scaling during Days 7 to 10 and on Day 12; no epidermal scaling was seen in males.

High-dose (1000 mg/kg/day): Among males very slight erythema was seen beginning on Day 3: in 1 animal on Days 3,4, 6,7, 14, 15, 16, 21; in 2 animals on Day 8; in 3 rabbits during Days 10-13; and in 4 animals on Day 9. No erythema was seen in males on Days 5, 17, 18, 19, and 20. Among females, very slight erythema was seen beginning on Day 1. This reaction was seen: in 1 female on Days 13, 14, 20, and 21; in 2 on Days 1, 4, and 5; in 3 during Days 6 to 11; and in 4 on Day 12. Epidermal scaling was noted in one male during Days 5 to 12 and on Day 17, and in one female on during Days 5, 6, 7 and 12, and in 2 on Days 8,9, and 10. No edema was seen in this group.

4. **Body Weight**

There were no biologically significant differences in body weight changes for males or females between treated and control groups. The transient weight loses observed in both sexes at all dose levels including the controls during the first week of treatment were attributed to acclimation of animals to wearing plastic collars. Consequently the statistically significant ($p < 0.05$) lower mean body weight change observed from Days 0 to 7 in males at the low-dose (-143.2 g) compared to controls (-13.6 g) was considered to be not toxicologically significant. Mean body weight an body weight gain were comparable between the treated and control groups from Days 7 to 14 and Days 14 to 21.

5. **Food Consumption**

Food consumption was generally comparable for all treated and control groups during the study.

6. **Hematology, Clinical Chemistry, and Urinalysis**

No biologically significant changes were seen in mean hematology, clinical chemistry, or urinalysis in treated groups when compared to appropriate corresponding control group values. The statistically significant differences seen in a few hematology and clinical chemistry data listed below:

- Decreases in leukocyte count and corrected leukocyte count in females at 10 mg/kg/day.
- Decreased absolute segmented neutrophil count in females at all treated groups at the pretreatment interval.
- Increases in creatinine values in males at 10 and 100 mg/kg/day groups.
- Decreases in cholesterol and triglyceride in females at 100 mg/kg/day.
- Increases in calcium values in males at 10 and 1000 mg/kg/day.
- Increases in total protein in males at 100 mg/kg/day.

These differences were not considered to be treatment-related due to the low magnitude of the change, presence of similar differences at the pretreatment intervals, and/or lack of dose response.

7. **Gross Pathology**

Gross pathological changes observed at necropsy were dark and/or edematous/swollen areas in the treated skin. The incidences were as follows:

	No. of males affected				No.of females affected			
Dose (mg/kg/day)	0	10	100	1000	0	10	100	1000
Dark areas	0	1	3	1	0	1	1	0
Edematous/swollen	0	0	0	0	0	1	0	0

Other gross pathological changes included one male in the control with a ruptured globe prior to terminal sacrifice; 1 female at the high dose with a ruptured globe after the terminal sacrifice; 1 male at the high-dose with dark areas on the glandular mucosa of the stomach; and 1 female at the mid-dose with dark areas of the lung.

8. **Organ Weight**

The mean absolute and relative kidney weights values were higher for males and females at the high dose; the difference, however, reached statistical significant ($p < 0.05$) only in the females. Other organ weight data were comparable between the treated and control groups.

9. **Histopathology**

There were no treatment-related histopathological changes were seen at any dose level. A similar frequency and severity of commonly seen spontaneous changes were seen in control and treated rabbits of both sexes.

IV. DISCUSSION

Repeated dermal application 2,4-Dichlorophenoxyacetic acid to male and female rabbits at doses 0, 10, 100, or 1000 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. 2,4-D acid was mildly irritating to the skin of rabbits inducing very slight erythema and epidermal scaling; in general, females were noted to have a higher incidence of dermal findings than males. No treatment-related effects were seen in survival, clinical signs, body weight gain, clinical, gross, or histopathology. A significant increase in mean absolute and relative kidney weights was observed for females at 1000 mg/kg/day group; however, the biological significance of this increase is not clear due to a lack of corroborating changes in clinical chemistry parameter or histopathological changes in the kidneys.

V. CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) for dermal irritation and systemic toxicity was 1000 mg/kg/day, the highest dose tested.

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

2. 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with 2,4-Dichlorophenoxyacetic Acid-2-ethylhexyl Ester (MRID No. 417353-05).

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with 2,4-D acid-2-ethylhexyl ester (2,4-D EH Ester).

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: 2,4-Dichlorophenoxyacetic Acid-2-ethylhexyl Ester
Purity: 98% pure
Acid equivalent: 62.8%
Lot No.: 04KF54479
Description: Pale yellow liquid

2. Test Animals

Species: Rabbit
Strain: Hra:(NZW)SPF
Sex: Males and females
Age at Initiation: Adult
Weight at Initiation: males - 2.4 to 2.7 kg;
females - 2.1 to 2.6 kg
Identification: Individual numbered ear tags and cage cards.
Acclimation: Approximately three weeks
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food: Certified High Fiber Rabbit Chow #5325 ad libitum
Water: Tap water ad libitum
Environment: Temperature- 64 to 76F; Humidity- 34 to 79%
Light / dark cycles: 12 hr. light/dark cycle

3. Study Design

Group No.	No. of Animals Males	Females	Test Material (mg/kg/day)[a]	Dose Volume (ml/kg/day)
1 (control)	5	5	0.0	0.00
2 (Low-dose)	5	5	16.3	0.01
3 (Mid-dose)	5	5	162.8	0.14
4 (High-dose)	5	5	1627.5	1.40

[a] Based on results of a 21-day pilot range-finding study.

4. **Test Material Formulation**

The test material was administered neat on a mg/kg/day basis. The appropriate volume of test material was applied to the shave area of the animals. Since test material was applied neat analyses were not required.

5. **Treatment**

Approximately 18 hours prior to dosing, the fur was clipped from dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. The appropriate volume of the test material was applied to the shaved area and covered with a 4 x 4" gauze patch and a Coban® wrap with a 4 x 4" plastic wrap secured in place with 1/2" two-sided sticky tape. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore a flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was wiped with dry gauze to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. **Experimental Procedures**

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Clinical signs	Days 0, 7, 14, and 21.
Body weight	Prior to initiation and on Days 7, 14, and 21.
Food consumption	Days 2,4,6,7,9,11,13,14,16, 18,20, and 21.
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit (HCT)	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Cell morphology
Mean cell hemoglobin (MCH)	Corrected leukocyte count
Mean cell hemoglobin concentration (MCHC)	

Clinical Chemistry

Calcium	Albumin
Inorganic phosphorus	Globulin
Chloride	Total protein
Sodium	Creatinine
Potassium	Total bilirubin
Glucose	Triglycerides
Alanine aminotransferase (ALT)	Total cholesterol
Aspartate aminotransferase (AST)	Alkaline phosphatase
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

7. Termination

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the kidneys, thyroid/parathyroid, testes with epididymides, and liver with drained gall bladder were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. Histopathology

The tissues listed above from all animals were trimmed and processed for histopathological evaluation.

9. Statistical Analyses

Appropriate statistical methods were employed for the analyses of body weights, body weight changes, food consumption, clinical pathology parameters and absolute and relative organ weight values.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Laboratories America, and the study protocol.

III. RESULTS

1. Survival

No mortality occurred during the study.

2. Clinical Signs

No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations

The dermal irritation scores are summarized in Tables 1 and 2 for males and females, respectively. Very slight erythema, epidermal scaling and, atonia were observed at the low-dose (16.3 mg/kg/day) while notable dermal irritation was observed at the mid-(162.8 mg/kg/day) and high-(1627.5 mg/kg/day) dose groups only. In general, edema was limited to the high-dose (1627.5 mg/kg/day) and its incidence and severity was greater for males than females. Edema was usually graded as very slight in severity (except for one male at the high dose where moderate edema was seen on Days 16 and 21). Additional dermal changes included skin thickening, epidermal scaling, fissuring (some with bleeding at the high dose) and atonia.

4. Body Weight

A reduction in mean body weight gain was observed in males at the low and mid dose from Days 0 to 21; the decrease reached statistical significance ($p < 0.01$) only at the mid dose. Males at all dose levels showed decreases in mean body weight gain from Days 14 to 21; however, the change was not statistically significant when compared to controls. Significantly ($p < 0.05$ or 0.01) lower mean body weight gain was observed in females at all dose levels from Days 14 to 21.

5. Food Consumption

Food consumption was generally comparable for all treated and control groups during the study.

Changes in Mean Body Weight (g) Changes in Rabbitss

Sex	Days	0 mg/kg/day	16.3 mg/kg/day	162.8 mg/kg/day	1627.5 mg/kg/day
Males	0 to 21	111.0 ± 103.8	-11.0 ± 64.3	-78.6** ±91.2	4.6 ± 72.9
	14 to 21	23.8 ± 51.3	-14.8 ± 41.2	-73.8 ± 52.3	-8.6 ± 60.7
Females	0 to 21	83.0 ± 156.9	92.0 ± 157.9	-2.6 ± 150.4	18.8 ± 69.1
	14 to 21	86.2 ± 32.6	-4.2* ±61.9	-37.6**± 52.3	9.0*± 32.0

* = $p \leq 0.05$; ** $p \leq 0.01$.

6. **Hematology, Clinical Chemistry, and Urinalysis**

No biologically significant changes were seen in mean hematology, clinical chemistry, or urinalysis in treated groups when compared to appropriate corresponding control group values. The statistically significant ($p < 0.05$) differences seen in a few hematology and clinical chemistry data considered to be spurious and/or incidental are listed below:

- Increase in mean cell hemoglobin concentration in males at 162.8 mg/kg/day group at Week 4.
- Decrease in creatinine level in females at all treated groups at Week 4; however, there was a slight increase in the mean value of the control group.

7. **Gross Pathology**

Gross pathology findings at the time of necropsy were noted primarily in the treated skin (dark, desquamated, edematous, and/or fissured) of the treated males and females.

8. **Organ Weight**

Mean absolute and relative kidney weights values were somewhat higher in males and females at the high dose; the difference, however, reached statistical significance ($p < 0.05$) only for relative weight in males. Other organ weight data were comparable between the treated and control groups.

9. Histopathology

Treatment-related histopathologic lesions of selected tissues was limited to treated skin; no compound related lesions were seen in the liver and kidneys. Lesions observed in the **treated and untreated skin** are summarized below:

No. of Animals with **Lesions in the Treated Skin**								
Sex	Males				Females			
Dose (mg/kg/day)	0	16.3	162.8	1627.5	0	16.3	162.8	1627.5
Lesion								
Acanthosis	0	0	3	5	0	0	3	4
Hyperkeratosis	0	0	2	4	0	1	3	5
Necrotic cellular debris on epidermal surface	0	0	1	5	0	0	1	2
Subepidermal edema	0	0	1	0	0	0	0	1
Inflammation, Chronic	1	1	3	5	2	1	3	5
Hemorrhage	0	0	2	0	0	0	0	2

No. of Animals with **Lesions in the Untreated Skin**								
Sex	Males				Females			
Dose (mg/kg/day)	0	16.3	162.8	1627.5	0	16.3	162.8	1627.5
Lesion								
Acanthosis	0	3	0	1	0	0	0	2
Inflammation, chronic	0	2	0	1	1	2	2	2
Subepidermal edema	0	0	1	0	0	0	0	0
Hemorrhage	0	2	0	0	1	0	0	0

IV. DISCUSSION

Repeated dermal application 2,4-Dichlorophenoxyacetic acid-2-ethylhexyl ester to male and female rabbits at doses 0, 16.3, 162.8, 1627.5 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. 2,4-D-2-ethylhexyl ester induced dermal irritating consisted of various degrees of erythema and edema whose incidence and severity were dose dependent. Additional dermal changes included skin thickening, epidermal scaling, fissuring (some with bleeding), and atonia. No treatment-related effects were seen in survival, clinical signs, food consumption or clinical pathology. Statistically significant depressions of body weight change were seen in treated females and nonsignificant depressions were seen in treated males during study week 3. A significant increase in mean relative kidney weights was observed for males at 1627.5 mg/kg/day group; however, the biological significance of this increase is not clear due to a lack of corroborating changes in clinical chemistry parameter or histopathological changes in the kidneys. Histopathologic changes observed in the treated skin included acanthosis, hyperkeratosis, and necrotic cellular debris on the epidermal surface; the incidence and severity of these lesions were increased in both males and females at the 162.8 and 1627.5 mg/kg/day groups. No histopathological lesions were seen in either sex at the low dose (16.3 mg/kg/day).

V. CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) was 16.3 mg/kg/day for dermal irritation and 1627.5 mg/kg/day for systemic toxicity. The lowest-observable-effect level (LOEL) for dermal irritation was 162.5 mg/kg/day.

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

Table 1. Dermal Irritation Scores In Male Rabbits Following Repeated Dermal Application of 2,4-D-ethylhexyl Ester.

SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

DAY :		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 16.3 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 162.8 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 4 1627.5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	4	3	3	3	3	3	3	2	3	3	3	2	1	2	0	0	0	0	0
	3	5	5	3	3	3	3	3	3	3	1	0	0	0	0	1	0	0	0	0	0	0	0
	4	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	1	2	2	2	2	2	2	3	2	2	2	3	4	3	5	5	5	5	5
	3	0	0	2	2	2	2	2	2	2	4	5	5	5	5	4	5	4	4	5	5	5	4
	4	0	0	5	5	0	1	1	0	0	1	1	3	2	2	5	3	2	2	5	4	5	4
WELL-DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1
	4	0	0	0	0	5	4	4	5	5	4	4	2	3	3	0	2	3	3	0	1	0	1
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	5	5	5	5	3	3	3	5	5	5	5	5	5	5	4	4	3	1	2	2	2	3
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	2	2	2	0	0	0	0	0	0	0	1	1	1	1	2	2	1	1
SLIGHT EDEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
MODERATE EDEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	4	4	5	5	4	0	0	0	0	0	1	1	0	0	0	0	0
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	4	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	4	0	0	0	0	0	:	2	1	1	4	1	5	5	5	1	4	5	5	4	5	5	5
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	2	2	1	2	0	0	0	0	1	0	0	0	0	0	0
ATONIA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	4	4	4	5	4	4	4	5	5	5	5	5	5	5	5

Table 2. Dermal Irritation Scores In Female Rabbits Following Repeated Dermal Application of 2,4-D-ethylhexyl Ester.

SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

DAY :	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																						
GROUP 1 0 MG/KG/DAY	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 16.3 MG/KG/DAY	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 162.8 MG/KG/DAY	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 4 1627.5 MG/KG/DAY	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SKIN/FUR																						
NO ERYTHEMA = 0																						
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	1	1	1	3	3	1	1	1	1	1	3	0	0	0	0	0	0	0	0
3	5	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	4	4	4	2	2	4	4	4	4	4	2	5	5	5	5	5	5	5	5
3	0	4	5	5	5	5	5	5	4	4	5	5	5	5	4	5	5	5	5	5	5	4
4	0	5	4	2	0	0	0	0	1	5	5	5	5	5	5	5	5	5	5	5	4	5
WELL-DEFINED ERYTHEMA = 2																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	1	3	3	2	3	3	4	0	0	0	0	0	0	1	0	0	0	0	0	0
MODERATE TO SEVERE ERYTHEMA = 3																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	2	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SEVERE ERYTHEMA = 4																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NO EDEMA = 0																						
1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	4	5
VERY SLIGHT EDEMA = 1																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
THICKENING																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	5	5	5	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0
EPIDERMAL SCALING																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3	0	0	0	0
4	0	0	0	0	0	0	0	1	2	0	0	0	0	1	5	5	5	5	2	4	2	5
FISSURING																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	1	2	3	3	5	5	5	5	2	5	5	5	5	1	0	0	1
FISSURING WITH BLEEDING																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
ATONIA																						
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	1	2	3	3	4	5	5	5	5	1	4	5	5	5	5	5	5	5

3. 21-Day Dermal Irritation and Dermal Toxicity Study in Rabbits with the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid(MRID No. 417353-06).

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a 21-day dermal toxicity study in rabbits with 2,4-D DMA.

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: **Dimethylamine salt of 2,4-Dichlorophenoxyacetic Acid**
Purity: 66.18% pure
Acid equivalent: 55.5%
Lot No.: 04FD31349
Description: Brown liquid
Vehicle: Distilled water
Lot No.: 1023913

2. Test Animals

Species: Rabbit
Strain: Hra:(NZW)SPF
Sex: Males and females
Age at Initiation: Adult
Weight at Initiation: males - 2.1 to 2.4 kg;
females - 2.2 to 2.5 kg
Identification: Individual numbered ear tags and cage cards.
Acclimation: Approximately two weeks
Health Status: Good
Housing: Individually in suspended wire mesh cages
Food:Certified High Fiber Rabbit Chow #5325ad libitum Water:
Water: Tap water ad libitum
Environment: Temperature- 61 to 76°F; Humidity- 24 to 66%
Light / dark cycles: 12 hr. light/dark cycle

3. **Study Design**

Group No.	No. of Animals Males	Females	Theoretical Dose Level (mg/kg/day)[a]	Actual Test Material (mg/kg/day)[b]
1 (control)	5	5	0.0	0
2 (Low-dose)	5	5	18.0	11.9
3 (Mid-dose)	5	5	180.1	119.2
4 (High-dose)	5	5	540.5	357.7

[a] Based on the results of a 21-day range-finding study, it was concluded that the doses for the full scale study would be a dose less than or equal to 18.0 mg/kg/day as the low dose, with an additional dose of 54.1 (mid-dose) and a high dose of 180.2 mg/kg/day. However, the mid- and high doses employed in the full scale study were higher than what was determined in the range finding study and no exaplanation was given for this divergence.

[b] Actual dosage levels are based on the purity of the test material (66.18%).

4. **Test Material Formulation**

The test material/vehicle suspension was prepared fresh weekly. The test material was shaken well (approximately 1 hour) prior to mixing to ensure homogeneity. The required amount of the compound was weighed, sufficient of distilled water was added, and the suspension was stirred on a magnetic stirrer for 3-4 minutes.

5. **Treatment**

Approximately 24 hours prior to dosing, the fur was clipped from dorsally and laterally from shoulder to rump to cover an area of approximately 10 % of the total body surface. The appropriate volume (dosing volume 1 mL/kg) of test material suspension was applied uniformly over the exposed area and covered with a 4 x 4 gauze patch and that was secured with a Coban® wrap, which was secured with an nonirritating adhesive tape. The animals were exposed for a period of 6 hours. To prevent possible ingestion of the test material, during the course of the 6 hour exposure period all animals wore a flexible plastic restraint collars. At the end of the exposure period, the dressings and collars were removed and the treated area was gently wiped (but not washed) with dry gauze to remove any remaining test material. Rabbits were treated for 6 hours/day, 7 days/week for 21 days.

6. Experimental Procedures

Parameter	Time measured
Mortality and Moribundity	Twice daily
Dermal irritation	Daily prior to treatment
Clinical signs	Days 0, 7, 14, and 21.
Body weight	Prior to initiation and on Days 7, 14, and 21.
Food consumption	Days 2,4,6,7,9,11,13,14,16, 18,20, and 21.
Hematology & Clinical Chemistry	Prior to initiation and at termination
Urinalysis	Termination

Hematology

Hematocrit (HCT)	Leukocyte count (WBC)
Hemoglobin (HGB)	Platelet count
Erythrocyte count (RBC)	Leukocyte differential
Mean cell volume (MCV)	Cell morphology
Mean cell hemoglobin (MCH)	Corrected leukocyte count
Mean cell hemoglobin concentration (MCHC)	

Clinical Chemistry

Calcium	Albumin
Inorganic phosphorus	Globulin
Chloride	Total protein
Sodium	Creatinine
Potassium	Total bilirubin
Glucose	Triglycerides
Alanine aminotransferase (ALT)	Total cholesterol
Aspartate aminotransferase (AST)	Alkaline phosphatase
Blood urea nitrogen	

Urinalysis

Appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	Glucose
Ketone	Microscopic examination of sediment

7. **Termination**

At termination, animals were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination and were subjected to a complete necropsy. At necropsy the kidneys, thyroid/parathyroid, testes with epididymides, and liver with drained gall bladder were weighed and organ-to-body weight ratios were calculated. Skin (treated and untreated), liver, kidneys, eyes, and target organs (those organs showing gross pathological changes) from all animals were fixed in 10% neutral-buffered formalin.

8. **Histopathology**

The tissues listed above from all animals were trimmed and processed for histopathological evaluation.

9. **Statistical Analyses**

Appropriate statistical methods were employed for the analyses of body weights, body weight changes, food consumption, clinical pathology parameters and absolute and relative organ weight values.

10. **Quality Assurance**

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the standard Operating Procedures of Hazleton Laboratories America, and the study Protocol.

III. RESULTS

1. Analytical Chemistry Data

As shown in Table 1 results of anlayses for stability (Day 0,3, 7, and 14), and routine analysis of Week 1 indicated adequate stability under the considtions of use.

2. Survival

Except for the two female rabbits at the high-dose that were sacrificed moribund on days 10 and 14, respectively, all animals survived until study termination.

3. Clinical Signs

The female rabbit at the high dose (Animal No. E50096) that was sacrificed moribund on day 10 was thin and languid in appearance on days 8 and 9 and prostrate on day 10. Another female rabbit at this dose (E50100) experienced immobility of the hindlegs due to injury/trauma to the lower back; necropsy of this animal confirmed lumbar spinal column fracture. Other clinical signs observed in control and treated animals included thin appearance, lacrimation, reddish mucus-like substance around hind legs, and diarrhea; none were considered to be treatment-related.

4. Dermal Observations

The incidences of dermal irritation are presented in Tables 3 and 4 for males and females, respectively. In the control group, one female exhibited very slight erythema on Days 1-4. In the low dose (18 mg/kg/day), very slight erythema was seen in one male from Days 2-6, and in one female from Days 2-11 and again on Day 21.

At the mid dose (180.1 mg/kg/day), very slight to well-defined erythema was seen on most males and females starting on Days 6 and 7. Other dermal effects observed in one or more males included thickening (Days 6-19), fissuring (Days 7-21), epidermal scaling (Days 6-17 and 21), and compound-like material at the treatment site (Days 4-21). Dermal observations in one or more females were thickening (Days 10-13) and (Days 15-21), epidermal scaling during Days (4-20), fissuring (Days 7-21), atonia (Days 7-11 and 17), and compound-like material at the treatment site during Days 3-21.

At the high dose (540.5 mg/kg/day), very slight to well-defined erythema was seen in all males and during Days 1-21 and the erythema increased to moderate to severe in one or two males from Day 9-21. Other dermal effects seen in one or more males included: thickening (Days 5-21), epidermal scaling (Days 6-21), fissuring (Days 6-21), fissuring with bleeding (Days 8-15), necrosis (Days 5-21), and compound-like material at the application site (Days 1-21). Among females, very slight to well-defined erythema was seen in all animals (Days 1-7) and again in a few animals from Days 17-21. Moderate to severe erythema was seen in a number of females from Days 7-21. Except for the very slight edema seen in one female during Days 1-3, no other edema was seen.Other dermal effects seen in one or more females were: thickening (Days 4-21), epidermal scaling (Days 7-14 and 17-21), fissuring (Days 6-21), fissuring with bleeding (Days 7-9), necrosis (Days 1-21), sloughing (Days 15-21), and compound-like material at the application site (Days 1-21).

5. **Body Weight**

No statistically significant differences were seen in mean body weight changes among treatment animals when compared to controls. The weight loss seen during the first few weeks of the study was attributed to the acclimation of the rabbits to wearing plastic collars.

6. **Food Consumption**

Food consumption was generally comparable between the treated and control animals.

7. **Hematology, Clinical Chemistry, and Urinalysis**

No treatment-related changes were seen in hematology, clinical chemistry, and urinalyses parameters.

8. **Gross Pathology**

Treatment-related gross pathological findings observed primarily in the treated skin of male and female rabbits included dark areas, desquamation, thickening, sores, eschar, compound residue, and/or fissuring. Gross pathological changes were also observed in the kidneys, stomach, bone, testes, urinary bladder and/or muscle; these findings were sporadic and not considered to be treatment related.

9. **Organ Weight**

A statistically significant ($p \leq 0.05$) increase was seen in the mean absolute kidney weights of males at 180.1 mg/kg/day group (17.6 g vs 14.8 g in controls); this increase was not considered to be treatment related since no significant changes in relative kidney weight and no corroborative histopathological changes were seen in the kidneys.

10. **Histopathology**

Histopathological lesions of the treated and untreated skins are summarized below. Treatment-related histopathological changes were limited to the treated skin of the mid and high-dose group animals. The treated skin sections of the low-dose were comparable to that of the controls.

No. of Animals with Lesions in the Treated Skin								
Sex	Males				Females			
Dose(mg/kg/day)	0.0	18.0	180.1	540.5	0.0	18.0	180.1	540.5
Acanthosis	0	0	5	5	0	0	5	4
Hyperkeratosis	0	0	5	5	0	0	5	4
Edema	0	0	3	2	0	0	1	4
Superficial crusting	0	0	1	5	0	0	1	4
Ulcer	0	0	2	0	0	0	0	0
Inflammation, cronic active	0	1	5	5	1	1	5	4

No. of Animals with Lesions in the Untreated Skin								
Sex	Males				Females			
Dose (mg/kg/day)	0.0	18.0	180.1	540.5	0.0	18.0	180.1	540.5
Acanthosis	0	0	1	0	0	0	0	1
Hyperkeratosis	0	0	1	0	0	0	0	1
Superficial crusting	0	0	0	0	0	0	0	1
Inflammation, subacute	2	1	1	2	5	2	1	2
Inflammation, Chronic active	0	0	0	0	0	0	0	10

IV. DISCUSSION

Repeated dermal application of Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid to male and female rabbits at doses 0, 18, 180.1, or 540.5 mg/kg, 6 hours/day, 7 days/week for 21 days did not result in severe systemic toxicity. Treatment-related dermal irritation consisted of various degrees of erythema and edema; the incidence and severity being dose dependent and generally greater in females than males. Additional dermal changes, also dose dependent, included skin thickening, epidermal scaling, fissuring (some with bleeding), necrosis, sloughing, compound-like material at treatment site, and atonia. Remarkable dermal changes were seen at 180.1 and 540.4 mg/kg/day dose groups, while only occasional, very slight erythema was seen at 18 mg/kg/day group; the latter finding was not considered to be toxicologically significant. No treatment-related effects were seen in survival, clinical signs, body weight, body weight gain, food consumption, or clinical pathology. The statistically significant increase in absolute kidney weight of male rabbits at 180.1 mg/kg/day group was not considered to be biologically significant since no changes were observed in relative kidney weight or in histopathology. Histopathological changes observed in treated skin at 180.1 and 540.5 mg/kg/day groups included acanthosis, hyperkeratosis, edema, superficial crusting (inspissated serum, necrotic cells and debris on epidermal surface) and chronic active inflammation. The treated skin lesions of the low-dose animals were comparable to controls.

V. CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) was 18 mg/kg/day for dermal irritation and 540.5 mg/kg/day for systemic toxicity. The LOEL for dermal irritation was 180.1 mg/kg/day.

VI. CORE CLASSIFICATION

Guideline; this study satisfies the requirements for a 21-day dermal toxicity study (82-3) in rabbits.

Table 1. Analysis of Dosing Solution.

Group	Dosage Level (mg/kg)		Corrected[a] Assayed Value (mg/ml)	Corrected[a] Percent Target %
Stability - Day 0				
1	0		ND	
2	18.0	A	11.44	96.1
		B	11.27	94.6
4	540.5	A	335.2	93.7
		B	329.6	92.2
Stability - Day 3				
1	0		ND	-
2	18.0	A	16.18	89.9
		B	15.76	87.6
4	540.5	A	474.1	87.7
		B	491.9	91.0
Stability - Day 7				
1	0		ND	-
2	18.0	A	17.12	95.1
		B	16.71	92.8
4	540.5	A	505.3	93.5
		B	491.8	91.0
Stability - Day 14				
1	0		ND	-
2	18.0	A	15.56	86.4
		B	15.74	87.4
4	540.5	A	467.1	86.4
		B	459.7	85.1
Routine Analysis - Week 1				
1	0		ND	-
2	18.0	A	17.48	97.1
		B	17.48	97.1
3	180.1	A	172.8	95.9
		B	169.8	94.3
4	540.5	A	509.6	94.3
		B	500.7	92.6

[a] The formulation was not adjusted for the given percent purity (66.18%); therefore, this value was used to determine the corrected assayed value.

- = Not applicable
ND = None detected

Table 2. Dermal Irritation Scores In Male Rabbits Following Repeated Dermal Application of Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid.

SEX: MALES — SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

		DAY: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 18.8 MG/KG/DAY		5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 188.1 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 4 560.5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	4	4	4	4	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	3	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	5	4	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	3	2	3	3	4	4	2	1	1	1	2	1	1	0	0	0	0	2	1	2	1
	4	0	1	1	2	2	3	3	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0
WELL DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	1	1	1	1	1	3	4	4	4	3	4	4	5	5	5	5	2	2	3	4
	4	0	0	0	0	0	2	3	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3
MODERATE TO SEVERE ERYTHEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	2	2	2	2
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	1	2	1	1	1	1	1	1	1	1	2	1	2	1	0	0
	4	0	0	0	0	0	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	1	4	5	5	5	5	5	5	4	3	2	2	0	0	0	1
	4	0	0	0	0	0	0	3	3	3	3	2	2	2	2	2	2	1	1	1	2	2	1
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	1	1	4	4	4	4	4	3	1	1	2	2	2	1
	4	0	0	0	0	0	0	1	3	3	3	3	5	4	4	4	4	4	5	5	5	5	5	5
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	1	2	2	1	1	1	1	1	0	0	0	0	0	0	0
NECROSIS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	2	2
COMPOUND-LIKE MATERIAL ON APPLICATION SITE	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	2	3	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	0	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 3. Dermal Irritation Scores In Female Rabbits Following Repeated Dermal Application of Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid.

008462

SEX: FEMALES

SUMMARY INCIDENCE OF DERMAL IRRITATION SCORES

	DAY :	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NUMBER OF ANIMALS EXAMINED																							
GROUP 1 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 2 10 0 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GROUP 3 100 1 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5
GROUP 4 500 5 MG/KG/DAY		5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3
SKIN/FUR																							
NO ERYTHEMA = 0	1	5	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	4
	3	5	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VERY SLIGHT ERYTHEMA = 1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1
	3	0	3	3	2	2	2	2	1	1	2	2	1	1	1	1	3	2	3	3	3	2	2
	4	0	2	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WELL DEFINED ERYTHEMA = 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	2	3	3	3	4	4	3	3	4	4	4	2	3	3	2	2	2	3	3
	4	0	2	2	2	5	5	5	1	0	0	0	0	0	0	0	0	0	2	2	2	2	2
MODERATE TO SEVERE ERYTHEMA = 3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	4	5	5	5	4	4	4	4	3	3	1	1	1	1	1
NO EDEMA = 0	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5
	4	5	4	4	4	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3
VERY SLIGHT EDEMA = 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THICKENING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1
	4	0	0	0	0	1	2	3	4	4	5	5	4	4	4	4	3	3	3	3	3	3	3
EPIDERMAL SCALING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	1	2	2	2	3	3	4	4	4	4	2	3	3	3	2	1	1	0
	4	0	0	0	0	0	0	0	2	2	2	1	1	1	1	1	0	0	1	1	1	1	1
FISSURING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	2	4	4	4	5	3	3	3	3	2	1	1	1
	4	0	0	0	0	0	0	1	3	3	3	5	4	4	4	4	3	3	3	3	3	3	3
FISSURING WITH BLEEDING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
ATONIA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	1	1	2	2	0	0	0	0	0	1	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NECROSIS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	1	1	1	2	2	2	4	5	5	5	4	4	4	4	3	3	3	3	2	1	1
SLOUGHING	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COMPOUND-LIKE MATERIAL ON APPLICATION SITE	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	4	0	0	5	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	5
	4	0	1	4	5	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. 7/3/91
Toxicologist, Review Section II, Toxicology Branch II/HED (H7509C)

Secondary Review by: K. Clark Swentzel 7/3/91
Section Head, Review Section II, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No.:417476-01
Caswell No.: 315
HED Project No.: 1-0621

Test Material: 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid)

Sponsor: Industry Task Force on 2,4-D Research Data

Study Number(s): 320-003 (Argus Research Laboratories)

Testing Facility: Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, Pa. 19044

Title of Report: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits

Author(s): Alan M. Hoberman, Ph.D.

Report Issued: December 12, 1990 (Final Report)

Study Dates: April 2, 1990 (first insemination)
May 4, 1990 (last cesarean section)

Bibliographic Citation: Hoberman, A.M., (1990) Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits. Study Number(s): 320-003 (Testing Facility) Argus Research Laboratories, Inc. 935 Horsham Road Horsham, Pa. 19044

Conclusions:

The test substance, 2,4-D Acid was administered by gavage to presumed pregnant rabbits at 0, 10, 30, and 90 mg/kg/day in 0.5% methylcellulose. Maternal toxicity was observed in two high dose does with ataxia. One high dose doe that aborted on day 21 had decreased bodyweight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to the touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group. There were no significant or dose related alterations in fetal development that could be attributed to treatment. The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to the control.

Core Classification: Core-Minimum

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 90 mg/kg/day
Developmental Toxicity NOEL = 90 mg/kg/day
Developmental Toxicity LOEL = >90 mg/kg/day

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine the dosage levels to be used in the main study. The study was sponsored by Industry Task Force on 2,4-D Research Data, and was performed by Argus Research Laboratories, Inc..

Study Title: Dosage-Range Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits (Pilot Study)

Study No.: Argus Research Laboratories, Inc., Protocol 320-003P

Date of Report: May 11, 1990

Date of Study: November, 2, 1989 (Insemination)

Author: Alan Hoberman, Ph.D. (Study Director)

Materials and Methods

The test substance 2,4-D Acid was administered by gavage days 6 through 18 of gestation at 0 (vehicle), 12.5, 25, 50, 100, and 200 mg (4 ml/kg/day, adjusted daily for bodyweight). The vehicle was 0.5% (w/w) methylcellulose. The doses were corrected for the 96.1% acid equivalent of the test substance. There were 4 animals per treatment group. On day 29 the animals were euthanized and necropsied. Corpora lutea were counted and the livers and kidneys weighed. The gravid uterus was excised and weighed. The number and location of implantation sites, early and late resorptions, and live and dead fetuses were recorded. Fetuses were weighed sexed, and examined for gross alterations.

Results

One doe in the 100 mg/kg group aborted and was sacrificed on day 22. Another doe in this group was sacrificed in the moribund condition. At the 200 mg/kg level, three does died. The does that were found dead, sacrificed, or aborted were found to have weight loss and decreased food consumption, impaired or lost righting reflex, decreased motor activity, ataxia, dyspnea, coldness to the touch, and/or a red substance present in the anogenital area or cage pan. A red-brown viscous fluid or orange-red fluid was present in the urinary bladder of the does that were found dead or sacrificed. With the exception of the does that aborted, were sacrificed moribund, or found dead there were no alterations in clinical signs, bodyweight or food consumption. There was no effect on maternal kidney weights. Absolute and relative liver weight was increased for the one doe surviving to the end of the

study. The litter of the one surviving doe at 200 mg/kg consisted of one viable fetus and 8 early resorptions, and the litter of the 100 mg/kg doe that aborted consisted of three early resorptions. All fetuses appeared normal at gross external examination.

Conclusions:

The dosage levels selected for the main study were 0, 30, 60, and 90 mg/kg.

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound:

The test article was stored at room temperature. Suspensions of the test article were prepared daily [as 0 (vehicle), 2.5, 7.5, and 22.5 mg/mL) and administered in a volume of 4 mL/kg. All dose concentrations were corrected for the 96.1% activity (purity) of the test substance.

The report references the pilot study for the homogeneity, concentration, and stability analyses. In addition, the report indicates that two 5 g samples of the test substance were sent to the sponsor for possible analyses, one at the beginning and one at the end of the dosing period. The results of this analyses are available in the sponsor's records.

Description:	tan granular powder
Lot No.:	909

Vehicle(s):

The vehicle was aqueous 0.5% methylcellulose (Sigma Chemical Co., Lot 88F0051). The vehicle was received in the form of white powder and stored at room temperature. The vehicle was prepared in R.O. deionized water. The water was analyzed for chemical and bacterial contaminants. After preparation, the vehicle was stored under refrigeration.

Test Animal(s):		
	Species:	Rabbit
	Strain:	New Zealand White [Hra:(NZW)SPF]
	Source:	Hazleton Research Products, Inc. Denver, PA
	Age:	approx. 5 mo. (upon receipt)
	Weight:	2.21 - 3.73 kg the day after arrival

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Acid when administered orally by stomach tube to female New Zealand White rabbits on gestation days 6 through 18, inclusive.

Mating

The females were artificially inseminated with semen from proven male breeders of the same strain, obtained from the same source. Each of these rabbits were given an i.v. injection of 20 USP Units of HCG (PREGNYL, Organon, Inc., Lot 0130289315) approx. 3 hrs.

prior to insemination. Semen was diluted with normal saline (Abbott Laboratories, Inc., Lot 34-201-JT) to achieve a concentration of 6.0 X 10^6 spermatozoa/0.25 mL for insemination. The day of insemination was considered day 0 of gestation. One quarter of the rabbits assigned to each group were inseminated each day. Four proven male breeders inseminated each group of females.

Group Arrangement:

One hundred four virgin female rabbits were received from the supplier. One animal upon exam by the Staff Veterinarian was found to be unacceptable. Another rabbit had an apparent fracture of the right rear leg and was sacrificed prior to assignment to the study. Eighty of these animals were assigned to the study (20/group) using a computer-generated weight ordered randomization procedure, after a 3 week acclimation period. The remaining 22 were reassigned to the general population.

Test Group	Dose Level (mg/kg)	Number Assigned
Vehicle Control	0	20
Low Dose	10	20
Mid Dose	30	20
High Dose	90	20

NOTE: Animal 16888 (vehicle control) was not dosed on day 9 due to repeated difficulties in placing the stomach tube. The technician deemed it necessary to refrain from further attempts so as to not jeopardize the health and life of the animal.

Animal Husbandry:

The study room had 370 sq. ft. of floor space and a minimum of 10 changes per hour of 100% fresh HEPA filtered (99.97%) air. The room temperature was 64 - 74° F; humidity was 35 - 65% during the study period. The animals were maintained on a 12 hour light/dark cycle. The animals were fed 180 g Purina Certified Rabbit Chow® # 5322. Local water that had passed through a R.O. membrane was also available ad lib via glass water bottles. Chlorine had been added to the water as a bacteriostat. The water samples contained from 0.0 to 0.6 ppm of chlorine.

Dosing:

The doses used in this investigation were selected on the basis of a dose range finding study.

All doses were in a volume of 4 mL/kg of bodyweight/day (adjusted daily for bodyweight). Suspensions of the dosing solutions were prepared daily. Triplicate 10 mL samples of each concentration

were reserved from the first and last time the dosage solution were prepared. Two of the samples were frozen and sent to Lancaster Laboratories, Inc. for analysis. The third frozen sample was kept at the facility as a backup. Two 5 g samples of the bulk test substance were also shipped to Lancaster Laboratories for possible analysis.

The dosing solutions for the pilot and main study were analyzed by Lancaster Laboratories. The detection limit was <0.05 mg/g. The results for the main study are as follows:

Theoretical Concentration of Dosing Solution:	Actual Range:
0 mg/mL	0
2.5 mg/mL	3 to 6%
7.5 mg/mL	3 to 4%
22.5 mg/mL	5 to 8%

The protocol indicates that the bulk test substance is stable at room temperature (stability of the test substance is on file with the sponsor).

Observations

Animals were observed for clinical signs several times during the acclimation period and on day 0 of pregnancy. The animals were checked for mortality at least twice daily. Clinical observations were performed three times each day during the dosing period (days 6-18), and once a day during the post dosing period.

Bodyweight was recorded on day 0, and days 6 through 29 of gestation. Food consumption was recorded daily.

Does were sacrificed on day 29 of gestation by i.v. administration of T-61® Euthanasia Solution. Thoracic and abdominal cavities were examined for gross lesions. In the event of gross lesions (except for parovarian cysts which are common in rabbits) the tissues were preserved in neutral buffered 10% formalin. A laparotomy was performed and the intact uterus was excised and weighed. Uteri that appeared nonpregnant were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded. Each fetus was removed from the uterus and individually identified with a tag, weighed, and observed for gross external alterations. Viable fetuses were sacrificed. Every fetus was examined to determine sex and soft tissue alterations. The brain was free-hand sectioned, and examined. Fetuses were then eviscerated, stained with Alizarin red S, and examined for skeletal changes. Skeletal specimens were stored in 80% glycerine with thymol crystals to retard fungal growth. Photographs were taken of all abnormal findings.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The section on statistical analyses is appended.

Compliance

A signed Statement of No Confidentiality Claim was provided dated December 15, 1990 (p 2).

A signed Statement of Compliance with EPA GLP's was provided that was dated December 15, 1990 (p 3).

A signed Quality Assurance Statement was provided dated December 12, 1990 (pp. 475 to 478).

A signed statement from the Chairman of the Technical Committee Industry Task Force II on 2,4-D Research Data, dated December 15, 1990 was provided, which indicated the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to this study. This study neither reportedly met or exceeded any of these criteria.

C. Results

1. Maternal Toxicity

Mortality

With the exception of two nonpregnant control does, all females survived to their scheduled sacrifice. One of the does was presumed to have died (on day 12) as a consequence of an intubation accident. Necropsy of the doe revealed hemorrhagic lungs. The cause of death for the other doe was not determined.

Clinical Observations

Alterations in clinical observations which were attributable to the test substance were observed in two high dose does. One doe (16937) had ataxia on days 16 to 19. Doe 16944 aborted on day 21. This doe presented the following clinical findings: dried feces, ataxia, decreased motor activity, loss of righting reflex, and extremities that were cold to the touch. Weight loss was observed in this animal after day 13. Feed consumption was decreased after day 5, decreases were more marked after day 12. Necropsy revealed a red substance in the anogenital area, a large gallbladder, and parovarian cysts.

Alopecia occurred in 2, 0, 3, and 2 does in the control, low, mid, and high dose groups. Rales occurred for 2, 3, 1, and 0 does in

the control, low, mid, and high dose groups. One control group doe had a red substance in the in the cage pan and was noted at necropsy to have a fluid filled uterus (the litter of this doe consisted of four early resorptions).

Bodyweight

Table I: Bodyweight Gains (kg)[a]

Dose (mg/kg):	Days 0-6	Days 6-19	Days 19-29	Days 0-29	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	0.19	0.22	0.25	0.66	-0.22	0.22
10.0	0.18	0.22	0.22	0.61	-0.19	0.20
30.0	0.17	0.24	0.25	0.66	-0.19	0.23
90.0	0.19	0.16	0.21	0.62	-0.24	0.17

1 = bodyweight gain during dosing period minus gravid uterus weight.
2 = corrected bodyweight = day 29 gestation bodyweight minus gravid uterine weight
a = Data extracted from (study number 320-003 tables 5 and 17 pp. 49 and 78-89)
(Some of the above values were calculated by this reviewer.)

The only time during which a reduction in maternal bodyweight gain was noted was during the dosing period (most notably during the initial phase of dosing). The average maternal weight gain was 100, 109.1, and 72.7% of the control value for the low, mid, and high dose groups, respectively. During the post-dosing period, the average maternal bodyweight gains were 88, 100, and 84% of the control values for the low, mid, and high dose groups. For the same respective groups the corrected bodyweight gain during the dosing period was 86.4, 86.4, and 109.1% of the control values; and the corrected bodyweight gain during the entire gestation period was 90.9, 104.5, and 77.3% of the control value.

Food Consumption

There were no significant differences in absolute (g/day) or relative (g/kg/day) maternal food consumption. Food consumption data excludes values for wet or spilled feed. Values for the does that aborted at the highest dose group were also excluded from the data.

Table II: Food Consumption Data (g/kg/day)[a]

Dose (mg/kg)	Days 0-6	Days 6-19	Days 19-29	Days 0-29
0	54.1	48.9	40.4	46.2
10.0	54.3	47.9	39.2	45.8
30.0	55.3	50.3	43.4	48.3
90.0	55.2	48.4	40.4	47.1

a = Data extracted from (study number 320-003 table 7 p.53)

Gross Pathological Observations

None of the necropsy findings were considered to be related to treatment. Some of the findings noted at necropsy have been presented above. Doe 16936 (of the high dose group) that aborted on day 24 was found to have mottled lungs. Doe 16944 (of the high dose group) that aborted on day 21 and was found to have a red substance present in the anogenital area also was found to have a large gall bladder and a parovarian cyst. Another doe of the high dose group was found to have the abdominal and thoracic cavities filled with clear fluid. Additional necropsy findings included parovarian cysts among all treatment groups.

Cesarean Section Observations

The does that aborted in the HDT were excluded from cesarean section observations. Table 9 of the report (p.56) indicates that the % resorbed conceptuses/litter was 6.4 for the control group. Apparently the value for doe 16876 was omitted from this mean. This doe had a litter that consisted of four early resorptions (litter 100% resorbed), which the author states is a relatively common event in rabbits. Historical control data is provided to support this statement.

There was a significant (dose-related) increase in the average percentage of live male fetuses in the 90 mg/kg group. The author reports that this value was even greater than that observed historically. When the author excluded litters containing only one sex, this finding was not statistically significant or dose-dependent.

No other findings were significantly altered relative to the control.

Table III: Cesarean Section Observations[a]

Dose (mg/kg):	0	10	30	90
#Animals Assigned	20	20	20	20
#Animals Inseminated	20	20	20	20
# (%) Pregnant	17(85.0)	18(90.0)	16(80.0)	18(90.0)
N	17	18	16	16
Maternal Wastage				
#Died	2	0	0	0
#Non pregnant	3	2	4	2
#Aborted	0	0	0	2
#Premature Delivery	0	0	0	0
Total Corpora Lutea	171	178	158	163
Corpora Lutea/Dam	10.0	9.9	9.9	10.2
Total Implantation	132	124	125	129
Implantation/Dam	7.8	6.9	7.8	8.1
Total Live Fetuses	119	120	109	116
Live Fetuses/Dam	7.0	6.7	6.8	7.2
Total Resorptions	13	4	16	13
Early	9	3	11	13
Late	4	1	5	0
Resorptions/Dam	0.8	0.2	1.0	0.8
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (g)	45.13	46.14	45.05	44.43
♂ fetuses	45.32	44.62	45.42	44.57
♀ fetuses	44.21	46.34	43.82	43.81
Preimplantation Loss(%)	22.5	29.6	19.4	19.3
Postimplantation Loss(%)	11.9	3.8	11.5	10.4
Sex Ratio (% Male)	52.8	54.4	59.4	71.2*

a Data extracted from (study number 320-003; tables 8, 9 pp. 55, 56 and tables 19 and 20 pp.102-109)

* Significantly different from vehicle control ($p \leq 0.05$)

(Some of the above values were calculated by this reviewer.)

2. Developmental Toxicity

Table IV: External Examinations

Dose (mg/kg):	0	30	60	90
Observations+				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
Head:				
Domed	0(0)	1(1)	0(0)	3(1)
Rhinocephaly	0(0)	1(1)	0(0)	0(0)
Palate:				
Cleft, Medial	0(0)	0(0)	0(0)	1(1)
Body:				
Kyphosis	0(0)	0(0)	0(0)	1(1)
Umbilical Hernia	0(0)	1(1)	0(0)	1(1)
Pale	0(0)	0(0)	0(0)	1(1)
Forelimb(s):				
Turned Inward	0(0)	0(0)	0(0)	2(1)
Paw, Left, Clenched	0(0)	0(0)	1(1)	0(0)
Hindlimb(s):				
Turned Inward	0(0)	0(0)	0(0)	3*(1)

Data extracted from (study number 320-003; table 11 pp. 58-59, and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence
* = significantly different from control ($p \leq 0.01$)

The fetal incidence of hindlimbs turned inward was significantly increased at 90 mg/kg. The fetal and litter incidence of domed head (and therefore, hydrocephaly) relative to the control was equal to that for hindlimbs turned inward also at 90 mg/kg. Fetus 16909-5 (of the 10 mg/kg group) had rhinocephaly, proboscis like nose above partially fused eyes and additional observations made upon soft tissue and skeletal exam. Fetus 16937-1 had a dome shaped head, a left turned fore- and hindlimb and other abnormalities observed with the soft tissue and skeletal exam. Fetus 16937-3 had a dome shaped head; the hindlimbs were bilaterally turned inward and other abnormalities observed with the soft tissue and skeletal exam. Fetus 16937-6 had a dome shaped head, medial cleft palate, kyphosis, fore- and hindlimbs were bilaterally turned inward and other abnormalities observed with the soft tissue and skeletal exam.

Table IV: Visceral Examinations

Dose (mg/kg):	0	10	30	90
Observations*				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
Brain:				
Extreme Dilation of Lateral Ventricles (Hydrocephalus)	0(0)	1(1)	0(0)	3(1)
Protrudes	0(0)	1(1)	0(0)	0(0)
Lungs:				
Intermediate Lobe, Agenesis	2(2)	7(5)	3(2)	5(2)
Gallbladder:				
Smaller than Normal	2(2)	0(0)	0(0)	3(3)
Larger than Normal	0(0)	0(0)	1(1)	1(1)
Liver:				
Protrudes	0(0)	1(1)	0(0)	1(1)

Data extracted from (study number 320-003; table 12 pp. 61-62 and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence

Upon soft tissue exam, fetus 16891-7 of the 10 mg/kg group, was found to have agenesis of the intermediate lobe of the lung and protruding liver. This fetus also exhibited findings in the skeletal exam. The three fetuses of the same litter (16937) that were observed to have domed head in the external exam were found to have hydrocephalus in the soft tissue exam.

Table IV: Skeletal Examinations

Dose (mg/kg):	0	10	30	90
Observations*				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
SKULL:				
Irregular Ossification	45(14)	42(17)	40(13)	45(15)
Nasal(s), Irregular Ossification:				
Internasal	0(0)	2(1)	1(1)	1(1)
Intranasal	0(0)	2(2)	3(3)	2(1)
Irregular Suture	4(3)	0(0)	0(0)	2(2)
Midline Suture Displaced	20(10)	16(10)	11(9)	22(11)
Small &/or Irregularly Shaped	0(0)	1(1)	1(1)	0(0)
Nasal(s)-Frontal(s) Irregular Suture	1(1)	0(0)	3(2)	3(3)
Frontal(s), Irregular Ossification:				
Interfrontal	3(3)	5(3)	4(3)	3(3)
Intrafrontal	2(2)	2(2)	4(4)	2(2)
Irregular Suture	26(11)	22(13)	20(12)	17(9)
Fused	0(0)	1(1)	0(0)	0(0)
Small and Irregularly Shaped	0(0)	1(1)	0(0)	0(0)
Parietal, Intraparietal	0(0)	1(1)	1(1)	0(0)
Zygomatics, Appear Broad	0(0)	1(1)	0(0)	0(0)
Maxillas, Short & Close-Set	0(0)	1(1)	0(0)	0(0)
Premaxillas, Absent	0(0)	1(1)	0(0)	0(0)
Palate, Inc. Ossified	0(0)	0(0)	0(0)	1(1)
Eye Sockets, Formed as One Below the Nasal	0(0)	1(1)	0(0)	0(0)
Anterior & Posterior Fontanelles Enlarged (Moderate)	0(0)	0(0)	0(0)	2(1)
Anterior & Posterior Fontanelles Enlarged (Marked)	0(0)	1(1)	0(0)	1(1)
Anterior Fontanelle, Additional Ossif.	0(0)	1(1)	0(0)	0(0)
Parietals, Contain Small Holes	0(0)	1(1)	0(0)	2(1)
Frontals, Contain Small Holes	0(0)	1(1)	0(0)	0(0)
HYOID, Ala(e), Angulated	4(3)	3(3)	4(3)	5(3)

Table IV: Skeletal Examinations- cont'd

Dose (mg/kg):	0	10	30	90
Observations*				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
VERTEBRAE:				
Cervical:				
Centrum Unilateral Ossification	0(0)	1(1)	0(0)	0(0)
Arch, Absent	0(0)	1(1)	0(0)	0(0)
Thoracic:				
Hemivertebra	0(0)	1(1)	0(0)	1(1)
Centrum, Unilateral Ossification	0(0)	1(1)	0(0)	1(1)
Lumbar:				
Hemivertebra	0(0)	0(0)	1(1)	0(0)
Caudal:				
Misaligned	1(1)	1(1)	1(1)	1(1)
INTERRELATED VERTEBRAL/ RIB MALFORMATIONS	0(0)	1(1)	0(0)	2(1)
RIBS:				
Fused	0(0)	1(1)	0(0)	3(1)
Split	0(0)	2(1)	0(0)	2(1)
Thickened	3(3)	0(0)	1(1)	0(0)
Wavy	0(0)	0(0)	0(0)	1(1)
STERNEBRAE:				
Fused	0(0)	1(1)	1(1)	0(0)
SCAPULAE:				
Alae, Irregularly-Shaped	0(0)	1(1)	0(0)	0(0)

Data extracted from (study number 320-003; table 13 pp. 63-69 and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence

Table 14, page 70 of the report indicates that there was a significant increase ($p \leq 0.01$) in the mean number of ossification sites in the lumbar vertebrae, and a significant decrease ($p \leq 0.01$) in the number of thoracic vertebrae at the 10 mg/kg level. Also at this level there was a significant reduction ($p \leq 0.05$) in the mean number of rib pairs. The report indicates that the author's facility identifies the number of thoracic vertebrae on the basis of the number of thoracic ribs present. Therefore, this observation was apparently related to the vertebral finding discussed above. Because these findings were not dose related and the incidences are within the historical control range, this observation is apparently unrelated to treatment.

The three fetuses in the high dose group of the same litter that were observed to have hydrocephalus and limbs that were turned inward were found to have enlarged anterior and posterior fontanelles. The parietals of two of these fetuses were found to contain small holes. The palate of the fetus found to have cleft palate was observed to be incompletely ossified. In addition, the ribs of this fetus bilaterally 1-12 were wavy and flat. Kyphosis in this fetus was confirmed upon the skeletal exam.

D. Discussion/Conclusions

a. Maternal Toxicity:

Two high dose does had ataxia. One high dose doe that aborted on day 21 had decreased bodyweight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to the touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group.

b. Developmental Toxicity:

i. Deaths/Resorptions:

There were no significant increases in fetal deaths or resorptions in any of the treatment groups.

ii. Altered Growth:

There were no significant alterations in fetal growth observed.

iii. Developmental Anomalies:

There was no increased incidence of developmental anomalies observed in this study that may be attributed to treatment.

iv. Malformations:

The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to the control.

E. Core Classification: Core-Minimum

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 90 mg/kg/day
Developmental Toxicity NOEL = 90 mg/kg/day
Developmental Toxicity LOEL = >90 mg/kg/day

END